PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 7/06 — A1

(11) International Publication Number: WO 94/09750

(43) International Publication Date: 11 May 1994 (11.05.94)

(21) International Application Number: PCT/GB93/02210

(22) International Filing Date: 27 October 1993 (27.10.93)

(30) Priority data:
9222772.7 30 October 1992 (30.10.92) GB

(71) Applicant *(for AU BB CA GB IE LK MN MW NZ SD only)*: UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).

(71) Applicant *(for all designated States except AU BB CA GB IE LK MN MW NZ SD)*: UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).

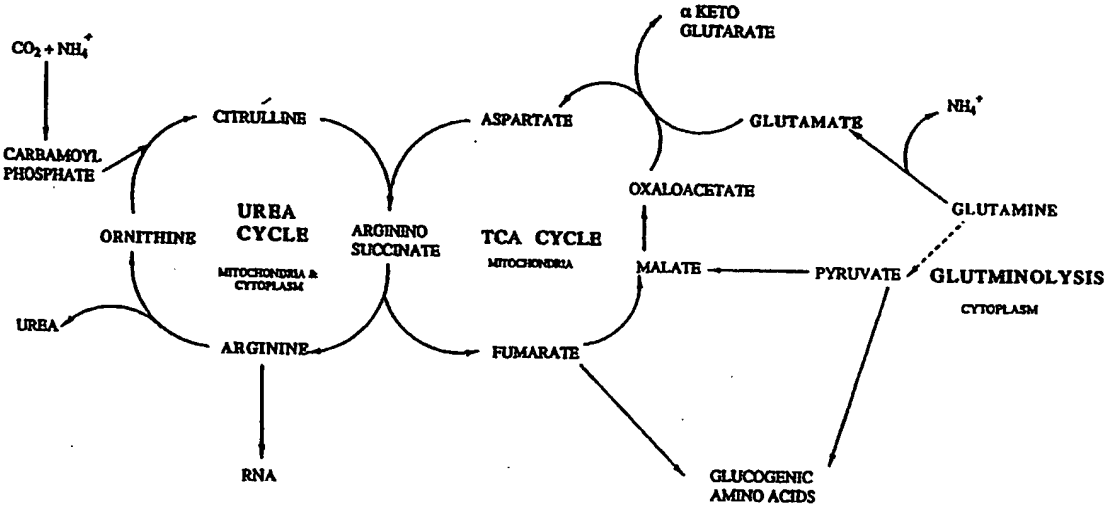
(72) Inventors: GIBSON, Walter, Thomas ; 24 Mackworth Drive, Finedon, Northampton NN9 5NL (GB). KEALEY, George, Terence, Evelyn ; 21 Lyndewode Road, Cambridge CB1 2HN (GB). WESTGATE, Gillian, ELizabeth ; 7 The Sidings, Pinetrees Village, Irthlingborough, Northamptonshire NN9 5RZ (GB).

(74) Agent: FORD, Michael, Frederick; Mewburn Ellis, 2 Cursitor Street, London EC4A 1BQ (GB).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: COSMETIC COMPOSITION



(57) Abstract

A cosmetic composition for topical application to mammalian skin or hair, especially to a bald or balding human scalp, contains a metabolic intermediate from the urea cycle, or a derivative of such an intermediate, as an active agent serving to promote growth of hair.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

COSMETIC COMPOSITION

FIELD OF THE INVENTION

The invention relates to a cosmetic composition for topical application to mammalian skin or hair for increasing or at least maintaining hair growth, especially terminal hair growth on the human scalp.

BACKGROUND TO THE INVENTION

In recent years, there has been great emphasis on the promotion, enhancement, or at least maintenance of normal hair growth on the human head, particularly with men whose hair has started to recede, as in male pattern baldness, or with both men and women, as hair becomes thinner with advancing age. To this end, the market in "hair restorers" or "baldness cures" is growing.

It is well established that in most mammals, including man, hair does not grow continuously, but undergoes a cycle of activity involving alternate periods of growth and rest. The hair growth cycle can be divided into three main stages, namely:

i) an active stage known as anagen, during which the hair follicle penetrates deep into the dermis with the cells of the bulb dividing rapidly and differentiating to form the hair,

ii) a regressive stage known as catagen, which is heralded by the cessation of mitosis, and during which the follicle regresses upwards through the dermis and hair growth ceases, and

iii) a resting stage known as telogen, in which the regressed follicle contains a small secondary germ with an underlying ball of tightly packed dermal papilla cells.

The metabolism of the hair follicle during growth is not well understood. It has been established on microdissected hair follicles that glucose is a major fuel which the hair follicle requires for normal growth and that glucose is not oxidised to any great extent, as most of it is converted to lactate. [See, for example, Adachi K, Uno H. Glucose Metabolism of Growing and Resting Hair Follicles. Am J Physiol (1968) 215, 1234-1239; and Philpott M P, Kealey T, Metabolic Studies on Isolated Hair Follicles. J Invest Derm (1991) 96, 875-879].

In more recent work, we have shown that surprisingly, the hair follicle metabolises very little lipid fuels. Instead, glutamine has emerged as a major fuel which yields as much energy to the hair follicle as does glucose. This work is described in EP 490 581 (Unilever) where we have shown that glutamine stimulates the linear rate of hair growth to a significant extent. This suggests that glutaminolysis is a very important metabolic process for hair growth.

As a result of further studies of glutaminolysis, we have now discovered that two thirds of the amine moiety derived from the conversion of glutamine to glutamate cannot be accounted for as ammonia. We therefore investigated its possible fate by examining other metabolic processes in human hair follicles, one of which, we hypothesised, is that the excess amine moieties were being disposed of by a functional urea cycle or part thereof, operating in the follicle.

The importance of the urea cycle for hair growth appears to reside in its ability to produce intermediates important for energy production in the follicle, which in turn enhance the efficiency of glutaminolysis. This is illustrated in Figure 1.

From this linked cycle, it can been seen that specific intermediates play an important part in promoting glutaminolysis. Thus, citrulline, which condenses with aspartate to form argininosuccinate, may also regulate the steps of arginine/urea synthesis by its availability, which would depend on oxaloacetate and in turn TCA cycle intermediates.

The availability of arginine may also be important for RNA and protein biosynthesis due to its being an essential amino acid. The significance of ornithine may be due to its role in the generation of arginine in the follicle rather than to urea. In the process, fumarate is produced which can feed into the TCA cycle and contribute to anaplerotic reactions. Without ornithine and arginine, the ammonia which results from a surplus of nitrogen caused by glutaminolysis may rise to toxic levels and prove fatal to cell function due its inhibition of transaminase reactions and hyperglutaminaemia.

We have now put this hypothesis to the test and discovered that supplementing a composition intended for topical application with metabolic intermediates of the urea cycle can cause an increase in hair growth in excess of that previously observed for glutamine.

The invention is accordingly concerned with the use of these intermediates in hair follicle metabolism, so as to increase or maintain hair growth.

DEFINITION OF THE INVENTION

Broadly, the invention provides a composition which is suitable for topical application to mammalian skin or hair for increasing, enhancing or maintaining hair growth, which composition comprises:

i) an effective amount of a metabolic intermediate of the urea cycle chosen from arginine, ornithine, citrulline, argininosuccinate, their salts, hydrosalts and precursors thereof, and mixtures thereof; and

ii) a cosmetically acceptable vehicle for the intermediate.

In another aspect the invention provides a method of increasing, enhancing or maintaining hair growth by use of such a composition. Use is by topical application especially to the bald or balding human scalp.

DISCLOSURE OF THE INVENTION

The Urea Cycle Intermediate

The invention is concerned with the utilisation of a metabolic intermediate which is part of the urea cycle, or a derivative of such a metabolic intermediate, and its topical application for the purposes of increasing or maintaining hair growth, particularly on the human scalp.

The urea cycle and its involvement in glutaminolysis (so important in hair growth) via the tricarboxylic acid

(TCA) cycle is shown in Figure 1. From this, four metabolic intermediates of the urea cycle, namely arginine, ornithine, citrulline and argininosuccinate have been selected as possessing the ability to promote linear hair growth to an extent which is significantly greater than glutamine itself.

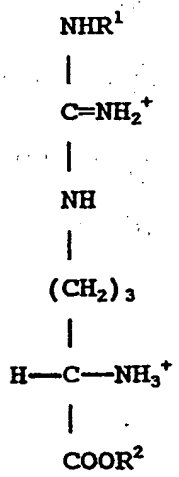
Each of these four metabolic intermediates can therefore be incorporated into a composition for topical application to skin or hair, in particular the scalp, for promoting or at least maintaining hair growth.

It is also possible and indeed is generally more convenient to employ simple derivatives such as salts and hydro salts, as appropriate, of these metabolic intermediates. A particularly preferred example of a salt is the sodium salt, and a preferred example of a hydro salt is a hydrohalide especially the hyrochloride derivatives of these intermediates.

It is further possible to employ other derivatives including acyl, ester and peptide derivatives of these metabolic intermediates. These too may be used as salts or hydrosalts.

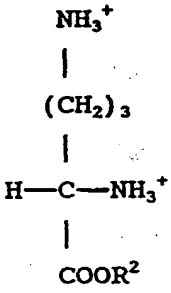
More particularly, derivatives as shown below in structures (1) to (4) can be employed.

Derivatives of arginine having the structure (1):



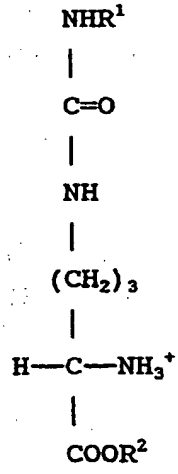
(1)

Derivatives of ornithine having the structure (2):



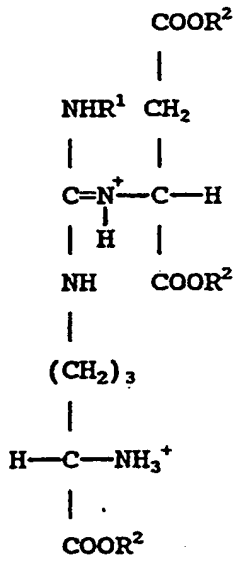
(2)

Derivatives of citrulline having the structure (3):



(3)

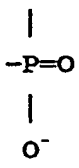
Derivatives of argininosuccinate having the structure (4):



(4)

where R^1 is chosen from:

(i) H-,

(ii) 

(iii) C_xH_y-,

(iv) amino acid residues, or substituted amino acid residues where any free $-NH_2$ group is modified to form a $-NHCOC_xH_y$ or a $-NHC_xH_y$ group, and/or any free -COOH group is substituted to form a $COOR^2$ group, and

(v) peptide residues comprising from 2 to 8 amino acid residues or substituted amino acid

residues, which are substituted as defined in (iv) above;

the amino acid residues or substituted amino acid residues, as herein defined, being derived from one or more of the following amino acids:

L-α-alanine
L-β-alanine
L-arginine
L-γ-amino butyric acid
L-asparagine
L-aspartic acid
L-citrulline
L-cysteine
L-cystine
L-3,4-dihydroxyphenylalanine (DOPA)
L-glutamine
L-glutamic acid
L-glycine
L-histidine
L-homoserine
L-hydroxylysine
L-hydroxyproline
L-isoleucine
L-leucine
L-lysine
L-methionine
L-ornithine
L-phenylalanine
L-proline
L-serine
L-threonine
L-N,N,N-trimethyl glycine (betaine)
L-tryptophan
L-tyrosine, and
L-valine;

and where each R^2 is chosen from:

(i) H^+,
(ii) alkali metal cations chosen from Na^+ and K^+,
(iii) NH_4^+ or alkanolammonium ions, and
(iv) C_xH_y-;

where x is a integer of from 1 to 22, and
y is an integer of from 3 to 45;

and where each $=NH_2^+$ and $-NH_3^+$ group is associated with an hydrohalide, where the halide is preferably Cl^- or Br^-.

Preferred examples of hydrohalide derivatives of urea cycle intermediates are:

arginine hydrochloride	(10)
ornithine hydrochloride	(11)

Preferred examples of alkali metal salt derivatives of urea cycle intermediates are:

sodium arginate	(12)
arginine phosphate, sodium salt	(13)
sodium arginosuccinate	(14)
potassium arginosuccinate	(15)

Preferred examples of dipeptide derivatives of urea cycle intermediates are:

L-α-alanylarginine hydrochloride	(15)
L-cystinylornithine	(16)
L-methionylcitrulline	(17)
L-arginylarginine	(18)
L-ornithylcitrulline	(19)
L-glutaminylarginine	(20)
L-glutaminylcitrulline	(21)

L-tyrosinylarginine	(22)
L-arginylmethionine	(23)
L-ornithylmethionine	(24)
L-citrullylmethionine	(25)
L-arginylaspartate	(26)
L-arginylglutamate	(27)
L-arginylleucine acetate salt	(28)
L-arginyllysine acetate salt	(29)
L-arginylphenylalanine acetate salt	(30)
L-ornithylaspartate	(31)

and their corresponding hydrchalides or salts, where appropriate.

Preferred examples of other derivatives of urea cycle intermediates are:

methylarginine dihydrochloride	(32)
arginine ethyl ester dihydrochloride	(33)
ethylcitrulline	(34)
n-propylornithine	(35)
n-octylarginine	(36)
n-octylcitrulline	(37)
n-octylornithine	(38)

The composition can comprise two or more urea cycle intermediates or derivatives thereof, as herein defined.

When the chosen urea cycle intermediate is citrulline, or a derivative thereof, it is preferably to include aspartate in the composition according to the invention, in view of the combination of citrulline and aspartate to from arginosuccinate, as is evident from Figure 1.

Likewise, when the chosen urea cycle intermediate is ornithine, or a derivative thereof, it is advantageous to include carbamoyl phosphate in the composition accordingly

to the invention, as carbamoyl phosphate promotes the formulation of citrulline from ornithine, as can also be seen from Figure 1.

The total amount of the urea cycle intermediate or derivative thereof present in the composition according to the invention is an amount which is sufficient to induce maintain or increase hair growth. This amount will depend on the effectiveness of the intermediate, some being more effective than others, but in general an amount of from 0.001 to 99%, usually from 0.01 to 30% by weight of the composition will provide an adequate concentration for application to the skin, particularly the scalp, which can then be repeated as necessary to promote hair growth.

The Cosmetically Acceptable Vehicle

The composition according to the invention also comprises a solid, semi-solid or liquid cosmetically and/or physiologically acceptable vehicle, to enable the urea cycle intermediate, or derivative thereof, to be conveyed to the skin at an appropriate dilution. The nature of the vehicle will depend upon the method chosen for topical administration of the composition. The vehicle can itself be inert or it can possess physiological or pharmaceutical benefits of its own.

The selection of a vehicle for this purpose presents a wide range of possibilities depending on the required product form of the composition. Suitable vehicles can be classified as described hereinafter.

It should be explained that vehicles are substances which can act as diluents, dispersants, or solvents for the urea cycle intermediate, or derivative thereof, which therefore ensure that they can be applied to and distributed evenly over the hair and/or scalp at an

appropriate concentration. The vehicle is preferably one which can aid penetration of the esters into the skin to reach the immediate environment of the hair follicle, compositions according to the invention can include water as a vehicle, and/or at least one cosmetically acceptable vehicle other than water.

Vehicles other than water can include liquid or solid emollients, solvents, humectants, thickeners and powders. Examples of each of these types of vehicle, which can be used singly or as mixtures of one or more vehicles, are as follows:

Emollients, such as stearyl alcohol, glyceryl monoricinoleate, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, eicosanyl alcohol, behenyl alcohol, cetyl palmitate, silicone oils such as dimethylpolysiloxane, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, cocoa butter, corn oil, cotton seed oil, olive oil, palm kernel oil, rapeseed oil, safflower seed oil, evening primrose oil, soybean oil, sunflower seed oil, avocado oil, sesame seed oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petroleum jelly, mineral oil, butyl myristate, isostearic acid, palmitic acid, isopropyl linoleate, lauryl lactate, myristyl lactate, decyl oleate, myristyl myristate;

Propellants, such as propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide;

Solvents, such as ethyl alcohol, methylene chloride, isopropanol, acetone, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol

monoethyl ether, dimethyl sulphoxide, dimethyl formamide, tetrahydrofuran;

Powders, such as chalk, talc, fullers earth, kaolin, starch, gums, colloidal silica sodium polyacrylate, tetra alkyl and/or trialkyl aryl ammonium smectites, chemically modified magnesium aluminium silicate, organically modified montmorillonite clay, hydrated aluminium silicate, fumed silica, carboxyvinyl polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate, ethylene glycol distearate;

The cosmetically acceptable vehicle will usually form from 10 to 99.999%, preferably from 10 to 99% and most preferably from 50 to 99% by weight of the emulsion, and can, in the absence of other cosmetic adjuncts, form the balance of the composition.

Activity Enhancer

The composition according to the invention can also optionally comprise an activity enhancer.

The activity enhancer can be chosen from a wide variety of molecules which can function in different ways to enhance the hair growth effects of the urea cycle intermediate. Particular classes of activity enhancers include (a) other hair growth stimulants, (b) penetration enhancers and (c) cationic polymers, whose presence can further improve the delivery of the ester through the stratum corneum to its site of action in the immediate environment of the hair follicle.

Some activity enhancers can also function as vehicles for the ester.

(a) Other Hair Growth Stimulants

i. Examples of other substances which themselves possess the ability to stimulate or increase hair growth include, for example:

Benzalkonium chloride
Benzethonium chloride
Phenol
Estradiol
Diphenhydramine hydrochloride
Chlorpheniramine maleate
Chlorophyllin derivatives
Cholesterol
Salicylic acid
Cystine
Methionine
Red pepper tincture
Benzyl nicotinate
dl-Menthol
Peppermint oil
Calcium pantothenate
Panthenol
Castor oil
Hinokitiol
Prednisolone
Resorcinol

Further substances which themselves possess the ability to increase the rate of terminal hair growth include:

ii(a) α-1,4 Esterified disaccharides described by Choay S.A. in EP-A 0 064 012; and

ii(b) Esterified oligosaccharides as described by Unilever in EP-A 0 211 610.

iii. Minoxidil glucuronides, as described by Unilever in EP 0 242 967.

iv. Minoxidil sulphates, as described by The Upjohn Co. in WO 86/04231.

v. Minoxidil, and other derivatives thereof as described by The Upjohn Co. in US 4 139 619.

vi. Ethylenediaminetetraacetic acid or salts thereof, as described by Redken Laboratories, Inc. in US 4 814 351.

vii. Direct proteoglycanase inhibitors, such as 1,10-phenanthroline, as described by Unilever in EP 0 277 428.

viii. Glycosaminoglycanase inhibitors, as described by Unilever in EP 0 277 428, such as D-Glucaro-1,4-lactone.

ix. Glycosaminoglycanase inhibitors, as described by Unilever in EP 0 277 428, such as N-acetylglucosamine.

x. Glycosaminoglycan chain cellular uptake inhibitors, as described by Unilever in EP 0 277 428, such as hexuronic acid and esters thereof.

xi. Chemical inhibitors of glycosidase activity, as described by Unilever in EP 0 334 586, chosen from lactams, such as D-glucaro-1,5-lactam.

xii. Chemical activators of protein kinase C enzymes, as described by Unilever in EP 0 334 585 chosen

from diacylglycerols, such as 1,2-Dioleoyl-sn-glycerol.

xiii. Glycosaminoglycanase inhibitors, as described by Unilever in EP 0 348 184, such as 6-methyl-glucaro-1,4-lactone.

xiv. Glycosaminoglycanase inhibitors, as described by Unilever in EP 0 348 184, chosen from acylated monosaccharides, such as 2-propionamido-2-deoxyglucose.

xv. Esters of pyroglutamic acid, as described by Lever Brothers Company in US 4 774 255, such as pyroglutamic acid n-hexyl ester and pyroglutamic acid n-octyl ester.

xvi. Hexosaccharic acids or an acylated hexosaccharic acids, or salts or esters thereof, as described by Unilever in EP 378 388, such as glucosaccharic acid, and its disodium salt.

xvii. Aryl-substituted ethylenes as described by Unilever in EP 403 238, such as 1,1-dicarboxy-2-(4-hydroxyphenyl)ethylene.

(b) Penetration Enhancers

As has been stated earlier, the presence of a penetration enhancer can potentiate the benefit of the urea cycle intermediate or derivative thereof by improving its delivery through the stratum corneum to its site of action in the hair follicle.

The penetration enhancer can accordingly function in a variety of ways. It can for example, improve the distribution of the urea cycle intermediate on the skin

surface or, it can increase its partition into the skin from the composition when applied topically, so aiding its passage to its site of action. Other mechanisms enhancing the benefit of the hair growth promoter may also be involved.

Examples of penetration enhancers include:

2-methyl propan-2-ol
Propan-2-ol
Ethyl-2-hydroxypropanoate
Hexan-2,5-diol
POE(2) ethyl ether
Di(2-hydroxypropyl) ether
Pentan-2,4-diol
Acetone
POE(2) methyl ether
2-hydroxypropionic acid
2-hydroxyoctanoic acid
Propan-1-ol
1,4 Dioxane
Tetrahydrofuran
Butan-1,4-diol
Propylene glycol dipelargonate
Polyoxypropylene 15 stearyl ether
Octyl alcohol
POE ester of oleyl alcohol
Oleyl alcohol
Lauryl alcohol
Dioctyl adipate
Dicapryl adipate
Diisopropyl adipate
Diisopropyl sebacate
Dibutyl sebacate
Diethyl sebacate
Dimethyl sebacate
Dioctyl sebacate

Dibutyl suberate
Dioctyl azelate
Debenzyl sebacate
Dibutyl phthalate
Dibutyl azelate
Ethyl myristate
Dimethyl azelate
Butyl myristate
Dibutyl succinate
Didecyl phthalate
Decyl oleate
Ethyl caproate
Ethyl salicylate
Isopropyl palmitate
Ethyl laurate
2-ethyl-hexyl pelargonate
Isopropyl isostearate
Butyl laurate
Benzyl benzoate
Butyl benzoate
Hexyl laurate
Ethyl caprate
Ethyl caprylate
Butyl stearate
Benzyl salicylate
2-hydroxypropanoic acid
2-hyroxyoctanoic acid,
Dimethyl sulphoxide
N,N-Dimethyl acetamide
N,N-Dimethyl formamide
2-Pyrrolidone
1-Methyl-2-pyrrolidone
5-Methyl-2-pyrrolidone
1,5-Dimethyl-2-pyrrolidone
1-Ethyl-2-pyrrolidone
Phosphine oxides
Sugar esters

Tetrahydrofurfural alcohol
Urea
Diethyl-m-toluamide, and
1-Dodecylazacycloheptan-2-one

(c) Cationic Polymers

As stated earlier, the presence of a cationic polymer can potentiate the benefit of the urea cycle intermediate or derivative thereof by improving its delivery to the hair and scalp. Examples of preferred cationic polymers include:

Guar Hydroxypropyltrimonium chloride
Quaternium-19
Quaternium-23
Quaternium-40
Quaternium-57
Poly(dipropyldiallylammonium chloride)
Poly(methyl-γ-propaniodiallylammonium chloride)
Poly(diallylpiperidinium chloride)
Poly(vinyl pyridinium chloride)
Quaternised poly (vinyl alcohol)
Quaternised poly (dimethylaminoethylmethacrylate); and mixtures thereof

The amount of activity enhancer, when employed in accordance with the invention, will normally be from 0.1 to 50%, preferably from 0.5 to 25% and most preferably from 0.5 to 10% by weight of the composition.

OPTIONAL SKIN BENEFIT MATERIALS AND COSMETIC ADJUNCTS

A particularly convenient form of the composition according to the invention is an emulsion, in which case an oil or oily material will normally be present, together with an emulsifier to provide either a water-in-oil

emulsion or an oil-in-water emulsion, depending largely on the average hydrophilic-lyophilic balance (HLB) of the emulsifier employed.

Oil or oily material

The composition according to the invention can optionally comprise one or more oils or other materials having the properties of an oil.

Examples of suitable oils include mineral oil and vegetable oils, and oil materials, such as those already proposed herein as emollients. Other oils or oily materials include silicone oils, both volatile and non-volatile, such as polydimethyl siloxanes.

The oil or oily material, when present for the purposes for forming an emulsion, will normally form up to 90%, preferably from 10 to 80% by volume of the composition.

Emulsifier

The composition according to the invention can also optionally comprise one or more emulsifiers the choice of which will normally determine whether a water-in-oil or and oil-in-water emulsion is formed.

When a water-in-oil emulsion is required, the chosen emulsifier or emulsifiers should normally have an average HLB value of from 1 to 6. When an oil-in-water emulsion is required, a chosen emulsifier or emulsifiers should have an average HLB value of >6.

Examples of suitable emulsifiers are set below in Table 1 in which the chemical name of the emulsifiers is

given together with an example of a trade name as commercially available, and the average HLB value.

Table 1

Chemical Name of Emulsifier	Trade Name	HLB Value
Sorbitan trioleate	Arlacel 85	1.8
Sorbitan tristearate	Span 65	2.1
Glycerol monooleate	Aldo MD	2.7
Glycerol monostearate	Atmul 84S	2.8
Glycerol monolaurate	Aldo MC	3.3
Sorbitan sesquioleate	Arlacel 83	3.7
Sorbitan monooleate	Arlacel 80	4.3
Sorbitan monostearate	Span 60	4.7
Poloxyethylene (2) stearyl ether	Brij 72	4.9
Poloxyethylene sorbitol beeswax derivative	G-1702	5
PEG 200 dilaurate	Emerest 2622	6.3
Sorbitan monopalmitate	Arlacel 40	6.7
Polyoxyethylene (3.5) nonyl phenol	Emulgen 903	7.8
PEG 200 monostearate	Tegester PEG 200 MS	8.5
Sorbitan monolaurate	Arlacel 200	8.6
PEG 400 dioleate	Tegester PEG 400-DO	8.8
Polyoxyethylene (5) monostearate	Ethofat 60-16	9.0
Polyoxyethylene (4) sorbitan monostearate	Tween 61	9.6
Polyoxyethylene (4) lauryl ether	Brij 30	9.7

Polyoxyethylene (5) sorbitan monooleate	Tween 81	10.0
PEG 300 monooleate	Neutronyx 834	10.4
Polyoxyethylene (20) sorbitan tristearate	Tween 65	10.5
Polyoxyethylene (20) sorbitan trioleate	Tween 85	11.0
Polyoxyethylene (8) monostearate	Myrj 45	11.1
PEG 400 monooleate	Emerest 2646	11.7
PEG 400 monostearate	Tegester PEG 400	11.9
Polyoxyethylene 10 monooleate	Ethofat 0/20	12.2
Polyoxyethylene (10) stearyl ether	Brij 76	12.4
Polyoxyethylene (10) cetyl ether	Brij 56	12.9
Polyoxyethylene (9.3) octyl phenol	Triton X-100	13.0
Polyoxyethylene (4) sorbitan monolaurate	Tween 21	13.3
PEG 600 monooleate	Emerest 2660	13.7
PEG 1000 dilaurate	Kessco	13.9
Polyoxyethylene sorbitol lanolin derivative	G-1441	14.0
Polyoxyethylene (12) lauryl ether	Ethosperse LA-12	14.4
PEG 1500 dioleate	Pegosperse 1500	14.6
Polyoxyethylene (14) laurate	Arosurf HFL-714	14.8
Polyoxyethylene (20) sorbitan monostearate	Tween 60	14.9
Polyoxyethylene 20 sorbitan monooleate	Tween 80	15.0
Polyoxyethylene (20) stearate	Myrj 49	15.0

Polyoxyethylene (20) stearyl ether	Brij 78	15.3
Polyoxyethylene (20) sorbitan monopalmitate	Tween 40	15.6
Polyoxyethylene (20) cetyl ether	Brij 58	15.7
Polyoxyethylene (25) oxypropylene monostearate	G-2162	16.0
Polyoxyethylene (20) sorbitol monolaurate	Tween 20	16.7
Polyoxyethylene (23) lauryl ether	Brij 35	16.9
Polyoxyethylene (50) monostearate	Myrj 53	17.9
PEG 4000 monostearate	Pegosperse 4000 MS	18.7

The foregoing list of emulsifiers is not intended to be limiting and merely exemplifies selected emulsifiers which are suitable for use in accordance with the invention.

It is to be understood that two or more emulsifiers can be employed if desired.

The amount of emulsifier or mixtures thereof, to be incorporated in the composition of the invention, when appropriate is from 1 to 50%, preferably from 2 to 20% and most preferably from 2 to 10% by weight of the composition.

Water

The composition of the invention can also comprise water, usually up to 90%, preferably from 5 to 80% by

volume. Water can function as the cosmetically acceptable vehicle.

Silicone Surfactant

The composition of the invention can also optionally comprise a high molecular weight silicone surfactant which can also act as an emulsifier, in place of or in addition to the optional emulsifier(s) already mentioned.

The silicone surfactant is a high molecular weight polymer of dimethyl polysiloxane with polyoxyethylene and/or polyoxypropylene side chains having a molecular weight from 10,000 to 50,000.

The dimethyl polysiloxane polymer is conveniently provided as a dispersion in a volatile siloxane, the dispersion comprising, for example, from 1 to 20% by volume of the polymer and from 80 to 99% by volume of the volatile siloxane. Ideally, the dispersion consists of a 10% by volume of the polymer dispersed in the volatile siloxane.

Examples of the volatile siloxanes in which the polysiloxane polymer can be dispersed include polydimethyl siloxane (pentamer and/or hexamer).

A particularly preferred silicone surfactant is cyclomethicone and dimethicone copolyol, such as DC 3225C Formulation Aid available from DOW CORNING. Another is laurylmethicone copolyol, such as DC Q2-5200, also available from Dow Corning.

The amount of silicone surfactant, when present in the composition will normally be up to 25%, preferably from 0.5 to 15% by weight of the emulsion.

Surfactants

The composition for use in the method according to the invention can be formulated as a shampoo and will then accordingly comprise one or more surfactants which are cosmetically acceptable and suitable for topical application to the hair. Examples of suitable shampoo surfactants are given below. When a composition is formulated to contain surfactant in a quantity of 4% by weight or more, it may prove desirable to use the metabolic intermediate in a concentration of more than 5% by weight, possibly more than 8% by weight. This may even prove desirable when surfactant is present in lower concentrations, such as 1% by weight or more.

Anionic Surfactant

The composition of the invention can comprise an anionic surfactant which is preferably chosen from alkyl sulphate, alkyl ether sulphate, alkyl sulphonate, alkyl aryl sulphonate, olefin sulphonate, acyl sarcosinate, acyl tauride, acyl isethionate, nonoalkyl sulphosuccinate, dialkylsulphosuccinate, acryl lactylate, acylated α-amino acid, alkyl carboxylate, monoalkyl phosphate and dialkyl phosphate.

Specific examples of anionic surfactants include:

alkyl sulphates, such as sodium lauryl sulphate [eg. EMPICOL CX available from Albright & Wilson], and triethanolamine lauryl sulphate [eg. EMPICOL TL40/T, available from Albright & Wilson].

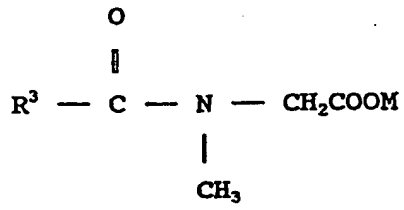
alkylether sulphates, such as sodium lauryl ether sulphate [eg. EMPICOL ESB70, available from Albright & Wilson].

alkyl sulphonates, such as sodium alkane (C_{13-18}) sulphonate [eg. HOSTAPUR SAS 30, available from Hoechst].

alkylaryl sulphonates, such as sodium alkyl benzene sulphonate [eg. TEEPOL CM44, available from Shell].

olefin sulphonates, such as sodium olefin sulphonate (C_{5-18}) [eg. HOSTAPUR OS, available from Hoechst].

acyl sarcosinates, having the structure: (51)



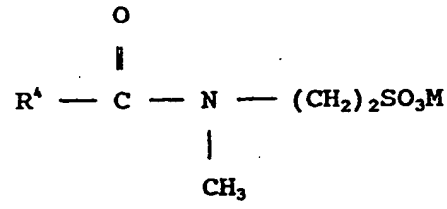
(51)

where R^3 is chosen from C_{5-14} alkyl, and

M is a counterion chosen from alkali metals, ammonium and substituted ammonium such as alkanolammonium.

An example of an acyl sarcosinate having the structure (51), is sodium lauryl sarcosinate [eg. HAMPOSYL L-95, available from Grace].

acyl taurides, having the structure (52):

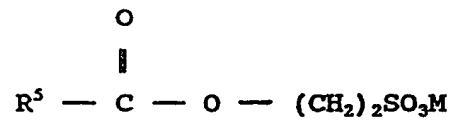


(52)

where R^4 is chosen from C_{8-18} alkyl

An example of an acyl tauride having the structure (52) is coconut methyl taurine [eg. FENOPEN TC 42, available from GAF].

acyl isethionates, having the structure (53):

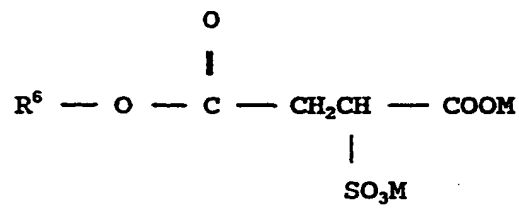


(53)

where R^5 is chosen from C_{8-18} alkyl.

An example of an acyl isethionate having the structure (53) is sodium acyl isethionate [eg. JORDAPON C1, available from Jordon].

monoalkyl sulphosuccinates, having the structure (54):



(54)

where R^6 is chosen from C_{10-20} alkyl.

Examples of monoalkyl sulphosuccinates having the structure (54) include:

sodium lauryl sulphosuccinate [eg. EMPICOL SLL, available from Albright & Wilson].

magnesium alkyl sulphosuccinate [eg. ELFANOL 616 Mg, available from AKZO].

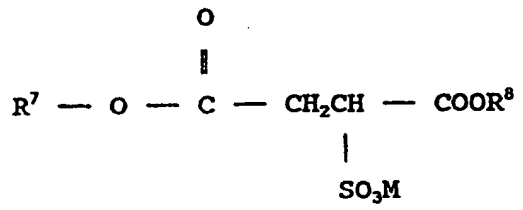
sodium lauryl ethoxysulphosuccinate [eg. EMPICOL SDD, available from Albright & Wilson].

coconut monoethanolamide ethoxysulphosuccinate [eg. EMPICOL SGG].

disodium lauryl polyglycolether sulphosuccinate [eg. SURTAGENE S30, available from CHEM-Y].

polyethyleneglycol sulphosuccinate [eg. REWOPOL SBFA 30, available from REWO].

dialkyl sulphosuccinates, having the structure (55):

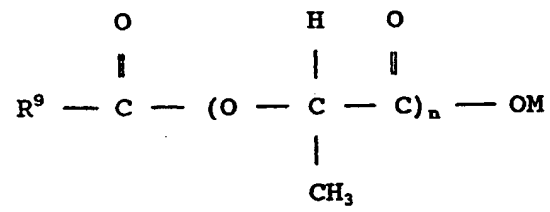


(55)

where R^7 and R^8 are the same or different, and are chosen from C_{6-14} alkyl.

An example of a dialkyl sulphosuccinate having the structure (55) is sodium dilauryl sulphosuccinate [eg. EMCOL 4500, available from Witco].

acyl lactylates, having the structure (56):



(56)

where R^9 is chosen from C_{6-16} alkyl,

and n is 1 or 2.

An example of an acyl lactylate having the structure (6) is decanoyl lactylate [eg. PATIONIC 122a, available from Patterson, CJ].

acylated α-amino acids, such as sodium lauroyl glutamate [eg. ACYLGLUTAMATE LS-11, available from Ajinomoto Co. Inc].

ethyl carboxlates, such as alkyl $C_{12-14}O(EO)_4OCH_2CO_2Na$ [eg. AKYPO RLM 38, available from Akzo].

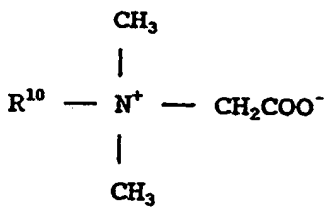
monoalkyl phosphates such as monolauryl phosphate, and dialkyl phosphates, such as dioctyl phosphate.

Amphoteric Surfactant

The shampoo compositions of the invention also comprise amphoteric surfactant. Suitable amphoteric surfactants are derivatives of aliphatic quaternary ammonium, phosphonium and sulphonium compunds, wherein the aliphatic radicals contain from 8 to 18 carbon atoms, and may be straight chain or branched, and further contain an anionic water-solubilising group, such as carboxyl, sulphonate, sulphate, phosphate or phosphonate.

Preferred amphoteric surfactants include:

Alkyl betaines, having the structure (57):

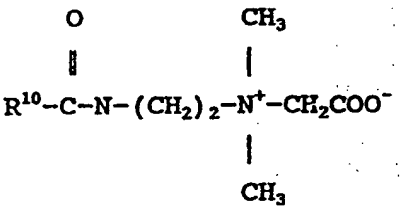


(57)

where R^{10} is C_{1-16} alkyl.

An example of an alkyl betaine having the structure (57) is lauryldimethyl betaine [eg. EMPIGEN BB, available from Albright & Wilson].

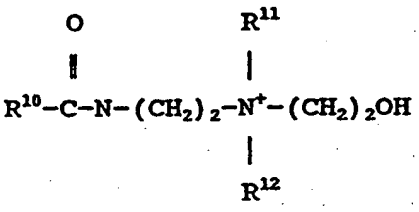
Alkylamidopropyl betines, having the structure (58):



(58)

An example of an alkylamidopropyl betaine having the structure (58) is cocamidopropyl betaine [eg. TEGOBETAIN L7, available from Goldschmidt).

Alkylamphoglycinates or Alkylamphopropionates having the structure (59):

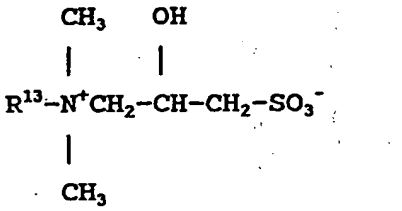


(59)

where R^{11} is chosen from H, CH_2COO^- and $(CH_2)_2COO^-$, and
R^{12} is chosen from CH_2COO^- and $(CH_2)_2COO^-$

Suitable examples of compounds (59) are cocoamphoglycinate (available from GAF), and cocoamphopropionate.

Sultaines, having the structure (60):



(60)

where R^{13} is chosen from C_{12-16} alkyl alkylamido groups.

An example of a sultaine having the structure (60) is cocamidopropylhydroxysultaine (eg. CYCLOTERIC BET-CS, available from Alcolac).

The most preferred amphoteric surfactant are lauryl dimethyl betaine and cocamidopropyl betaine.

Such amphoteric surfactants can contribute to the foaming of the shampoo of the invention, while ameliorating the harshness of the anionic surfactant.

Nonionic Surfactant

The shampoo composition of the invention can also comprise alkoxylated or glycosidic nonionic surfactant having an HLB of 8 or more. Above this value nonionics generally form clear isotropic solutions in combination with the other surfactants in the ranges defined above. Preferred nonionic surfactants are polyoxyethylene alkyl esters and polyoxyethylene alkyl ethers and alkyl polyglycosides.

A suitable example of a polyoxyethylene alkyl ester is that having the CTFA designation Polysorbate 80 which is a mixture of oleate esters of sorbitol and sorbitol anhydrides, condensed with approximately 20 moles of ethylene oxide. Also suitable is Polysorbate 20 which is a mixture of laurate esters or sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide.

Polysorbate 80 and Polysorbate 20 are available commercially as TWEEN 80 and TWEEN 20 respectively, from ICI Americas.

Also suitable for use in the compositions of the invention is the polyethylene glycol ether of C_{9-11} alcohol

with an average of 8 ethoxy units, which is available commercially as NONIDET LE-8T or as SYNPERONIC 91-8T, and the polyethylene glycol ether of C_{12-15} alcohol with an average of 9 ethoxy units which is available commercially as DOBANOL 25-9.

Particularly useful alkyl polyglycosides include the glycosides of glucose or glucose oligomers where the alkyl chain can be C_{8-16} and the average number of glucose units is 1 to 2. A suitable example is ORAMIX NS 10 which is the glucoside of C_{10-12} fatty alcohol with an average of about 1.5 glucose units.

The amount of surfactant that can be present in the composition accordingly to the invention is up to 30%, preferably from 1 to 20% by weight of the composition.

Other Cosmetic Adjuncts

Examples of conventional adjuncts which can optionally be employed include antioxidants, such butyl hydroxy toluene; sunscreens, such as octyl methoxycinnamate and butylmethoxy di-benzoyl methane; film forming agents, such as perfluoropolymethylisopropyl ether humectants, such as glycerol, sorbitol, 2-pyrrolidone-5-carboxylate, dibutylphthalate, gelatin, polyethylene glycol, such as PEG 200-600; buffers, such as lactic acid together with a base such as triethanolamine or sodium hydroxide; waxes, such as beeswax, ozokerite wax, paraffin wax: plant extracts, such as Aloe vera, cornflower, witch hazel, elderflower, cucumber; thickeners; activity enhancers; colourants; and perfumes. Cosmetic adjuncts can form the balance of the composition.

Preservation of the Composition

The composition according to the invention is preferably preserved in such a manner that it will enjoy an extended shelf life following manufacture and prior to sale and use. Ideally the composition will have an indefinite shelf life.

It is accordingly apparent that the urea cycle intermediate is likely to be prone to attack by bacteria, moulds and fungi and other microbial influences, particularly at pH values near that of the skin that characterise the preferred composition. The shelf-life of the composition can therefore be unacceptably short due to the biodegradation of the precursor unless steps are taken to preserve the composition.

In order to be preserved, the composition should preferably be free, or substantially free, from viable microbial contaminants that are capable of resulting in microbial spoilage of the composition, and/or biodegradation of the precursor prior to topical application of the composition to mammalian skin or hair. It is to be understood, however, that the invention is also concerned with compositions, as herein defined, which may contain viable but dormant microorganisms, such as bacterial spores, provided that the conditions of preservation do not result in substantial proliferation of the microorganisms prior to use of the composition.

Examples of the methods that can be employed to achieve preservation of the composition, includes the following:

(i) Sterilisation

The composition according to the invention can be preserved by sterilisation to remove or kill substantially all viable microbial contaminants. This can be achieved for example by irradiation using a lethal dose of gamma rays, by heat sterilisation or by ultrafiltration using techniques that are well established in the pharmaceutical industry.

(ii) Chemical Preservative

The composition according to the invention can also be preserved by including in it a chemical preservative which functions to prevent the growth of or kill bacteria, fungi or other microorganisms.

Examples of chemical preservatives include ethanol, benzoic acid, sodium benzoate, sorbic acid, potassium sorbate, sodium propionate and the methyl, ethyl, propyl and butyl esters of p-hydroxybenzoic acid. The amount of chemical preservative that can be incorporated in the composition according to the invention will generally be from 0.05 to 5%, preferably from 0.1 to 2% by weight, the amount chosen being sufficient to arrest microbial proliferation.

(iii) Water activity depressants

The composition according to the invention can also be preserved by the inclusion of a water activity depressant such as glycerol, propylene glycol, sorbitol, sugars and salts, for examples alkali metal halides sulphates and carboxylates. When employing a water activity depressant, sufficient should be incorporated in the composition according to the invention to reduce the water activity (α_w) from 1 to < 0.9, preferably to < 0.85 and most preferably

< 0.8, the lowest of these values being that at which yeasts, moulds and fungi will not proliferate.

PRODUCT FORM AND PACKAGING

The topical skin and/or hair treatment composition of the invention can be formulated shampoo or hair conditioner, or as a liquid or gel, or as a lotion having a viscosity of from 4,000 to 10,000 mPas, a fluid cream having a viscosity of from 10,000 to 20,000 mPas or a cream having a viscosity of from 20,000 to 100,000 mPas, or above.

The composition can be packaged in a container to suit its viscosity and intended use by the consumer. For example, a shampoo, conditioner, liquid, gel, lotion or fluid cream can be packaged in a bottle or a sachet or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar.

The invention accordingly also provides a closed container containing a cosmetically acceptable composition as herein defined.

Process

The invention also provides a process for preparing a hair growth composition which comprises the steps of mixing an effective amount of a urea cycle intermediate, as herein defined, together with a cosmetically acceptable carrier for the intermediate.

Use of the urea cycle intermediate for increasing or maintaining hair growth

The invention accordingly also provides for the use of a urea cycle intermediate, as herein defined, for topical application to mammalian skin or hair for increasing or maintaining hair growth.

The compositions according to the invention are primarily intended for topical application to the scalp of the human subject, particularly where the head is already bald or balding, in order to convert vellus hair to growth as terminal hair, or to increase the rate of growth of terminal hair. The compositions can also be applied prophylactically to the hair and hence the scalp to reduce or prevent the onset of baldness, by maintaining the hair in a healthy, growing state, with less that usual hair loss.

The amount of the composition and the frequency of application to the hair and/or scalp can vary widely, depending on personal needs, but it is suggested as an example that topical application of from 0.1 to 5g daily containing from 0.00001 to 1g of a selected urea cycle intermediate over the period of at least six months will in most cases result in an improvement in hair growth.

EVALUATION OF EFFICACY OF THE UREA CYCLE INTERMEDIATES USING THE IN VITRO HAIR FOLLICLE GROWTH TEST

The effect of compounds on hair growth was assessed using an in vitro test which measures the elongation of isolated human hair follicles in a culture medium.

Isolation of the hair follicle from skin

This test includes the important step of isolating hair follicles having an undamaged hair bulb from human skin, for example, facelift skin, by microdissection. The method employed is described by Philpott et al; in J Cell Sci 97, 463, (1990).

The critical step of separating the hair follicle with intact undamaged hair bulb from the subcutaneous fatty tissue in which it is situated accordingly involves severing the hair shaft of the follicle at a point below the epidermis of skin surface, so as to leave the hair bulb intact and undamaged while still bearing a portion of the hair shaft.

Preferably, the hair shaft of the follicle is severed at the dermal-subcutaneous fat interface.

Any suitable cutting instrument can be employed to sever the hair shaft in this manner, but a keratotome or a scalpel are preferred.

The hair bulb with a hair shaft stump attached is then isolated from the skin by mechanically separating the hair from loosely adhering subcutaneous fat which normally surrounds the hair bulb. This is achieved after the dermis or upper layer of the skin has been separated and removed, to avoid damaging the hair bulb as it is pulled away.

The hair bulb together with hair shaft stump attached, is then transferred in an otherwise undamaged and fully functioning, viable state to a nutrient medium.

Culture of the isolated hair follicle

The hair follicles isolated by the technique described herein are transferred to a suitable culture medium for subsequent testing of substances that can then influence their future development.

The procedure now to be described represents a preferred method of culture and testing of hair growth.

In accordance with the preferred method of culture, isolated hair follicles, obtained from facelift skin are maintained in 1 ml of Williams E medium, supplemented with L-glutamine (2mM), insulin (10 µg/ml), hydrocortisone (10ng/ml) and antibiotics, either with or without a test hair growth substance (urea cycle intermediate). The medium was incubated at 37°C in an atmosphere of 5% CO_2 + 95% air in individual wells of a 24 multiwell dish (Corning), which permits detailed measurements to be made of the length of individual hair follicles.

Williams E medium is available from FLOW Laboratory under Catalogue No. 12-502. The formula of Williams E medium is described by Williams GM, et al., in Experimental Cell Research 69 (1971) on page 106.

Daily growth rate and cumulative growth for each follicle were calculated by measuring the change in length of the follicles each day using a microscope fitted with an eyepiece graticule and from this the average of all the follicles was calculated.

Evaluation of Results

The response of an isolated hair follicle to a test substance, can accordingly be assessed by measuring the increase in length, if any, in the presence of a test substance against a control.

The in vitro method described herein was used to assess the effect of three urea cycle intermediate, namely arginine hydrochloride, ornithine hydrochloride and citrulline, when used in the presence of glutamine, compared with glutamine alone, to demonstrate the superiority of the urea cycle intermediates over glutamine alone.

The results obtained are summarised in the following Table 2 below.

SUBSTITUTE SHEET

Table 2

Urea Cycle Intermediates in Williams E Medium and L-Glutamine	Amount of Hair Growth in 5 Days (mm)	Average Daily Rate of Hair Growth (mm/day)	% Increase in Rate of Hair Growth (P=probability) as calculated by Student's t test)	Total Number of Observations
EXPERIMENT A GLUTAMINE 2mM[1]	1.203 ± 0.07	0.241	-	18[2]
GLUTAMINE 2mM ORNITHINE 1mM	1.580 ± 0.06	0.316	31.1% (P<0.001)	18[2]
GLUTAMINE 2mM ORNITHINE 5mM	1.491 ± 0.063	0.298	23.6% (P<0.001)	18[2]
GLUTAMINE 2mM ARGININE 1mM	1.430 ± 0.08	0.286	18.7% (P<0.0125)	18[2]
GLUTAMINE 2mM ARGININE 5mM	1.340 ± 0.080	0.268	11.2%[4]	18[2]
EXPERIMENT B GLUTAMINE 2mM[1]	1.374 ± 0.026	0.275	-	57[3]
GLUTAMINE 2mM CITRULLINE 5mM	1.474 ± 0.026	0.295	7.3% (P<0.005)	54[3]

Notes on Table 2

1. Controls (without added urea cycle intermediate).

2. Results based on observation on the growth of hair follicles taken from facelift skin of three patients. For each treatment, follicles from each patient were tested in accordance with the method described herein, making a total of 18 treatments in all.

3. Results based on observation of growth of hair follicles taken from facelift skin from five patients. For each treatment, follicles from each patient were tested in accordance with the method described herein, making a total of 57 treatments for the control medium (glutamine alone), and 54 treatments for the medium supplement with citrulline.

4. Not significant as calculated Student's t test.

The results summarised in Table 2 above indicate the significant increase in hair growth, as compared with glutamine alone (control), that can be achieved with each of the urea cycle intermediates in the presence of glutamine, as promoters of the rate of hair growth. This confirms that urea cycle intermediates promote or at least maintain hair growth.

In a similar experiment, using Williams E medium as the control, and Williams E medium supplemented with 1mM arginine as the test medium, it was found that there was some variation in the percentage increase in hair growth for follicles from three different patients. The percentages were 7%, 3.5% and 6% increase in hair growth compared to controls from the same patients. The average of 5.5% increase in hair growth was clearly statistically significant.

Like experiments was carried out using 1mM ornithine and 1mM citrilline as the supplement to the Williams E

medium. The results with follicles from each patient were statistically significant and averaged 7.4% for citrulline and 6.4% for ornithine.

A test procedure for total protein synthesis utilised a similar culture of isolated follicles. The follicles were cultured in arginine-deficient Williams E medium supplemented so as to contain 0.287mM, 0.574mM or 1.435mM arginine, for two days. Incubation was then continued for a further three hours in the presence of ^{14}C labelled leucine as well as the same concentration of arginine as before.

After incubation, the follicles were removed from the radioactive media, and washed with 3 x 1ml of PBS supplemented with 10mM unlabelled leucine, to displace non-covalently bound ^{14}C-leucine. They were then homogenised in 1ml of ice-cold 0.1M K_2EDTA, pH 12.3, in a hand-held glass/glass homogeniser. The homogenate was left at 4°C for 30 min. to release and dissolve the protein. Cell debris was removed by centrifugation, and 110μl samples of the supernatant removed for total protein determination by the Bio-Rad assay [based on Bradford, Anal.Biochem., 72, 248 (1976)]. To the remaining supernatant was added 0.5ml of ice-cold 15% TCA, and this was left at 4°C for 15 min. to precipitate out the protein. The precipitate was collected, and washed with 4 x 5ml of 15% TCA, under vacuum onto Whatman GF/C (2.4cm) filters, which had been pre-washed with PBS supplemented with 10mM unlabelled leucine. Finally, the filters were dried and their radioactivity determined by liquid scintillation spectrometry. The cell debris was solubilised in 0.5ml of Soluene, and counting its radioactivity showed that less than 15% of incorporated radioactivity was discarded in the cell debris pellet.

The results were:

Arginine concentration (mM)	Leucine uptake (pmol/hour/follicle)
0.287	80
0.574	93
1.435	110

This showed that the arginine was enhancing protein synthesis in the hair follicles.

EXAMPLES

The invention is illustrated by the following examples, each of which provides a composition comprising one or more specific metabolic intermediates of the urea cycle or derivatives thereof in in accordance with the invention. Selected metabolic intermediates are indicated by the structure numbers listed earlier in this specification.

Example 1

This Example illustrates a lotion according to the invention which is suitable for topical application to the skin in order to enhance hair growth.

The lotion has the following formulation:

	% w/w
Intermediate (10)	1
ethanol	99
perfume	q.s.

Example 2

This Example illustrates a hair tonic which is suitable for application to hair or scalp.

The hair tonic has the following formulation:

	% w/w
intermediate (11)	2
ethanol	49
water	49
perfume	q.s.

Example 3

This Example also illustrates a lotion which is suitable for topical application to the scalp.

The lotion has the following formulation:

	% w/w
Intermediate (12)	3
propan-2-ol	10
ethanol	87
perfume	q.s.

Example 4

This Example also illustrates a hair tonic which is suitable for application to hair or scalp.

The hair tonic has the following formulation:

	% w/w
Intermediate (13)	3
ethanol	40
water	57
perfume	q.s.

Examples 5 to 8

The following formulations represent lotions which can be used topically in the treatment of bald or balding male or female heads.

	% w/w			
	5	6	7	8
Hydroxyethyl cellulose	0.4	-	0.4	-
Absolute ethanol	25	25	25	25
Propane-1,2-diol	-	-	38.4	38.4
Butane-1,3-diol	38.4	38.8	-	-
Paramethyl benzoate	0.2	0.2	0.2	0.2
Intermediate (14)	5	-	-	-
Intermediate (15)	-	4	-	-
Intermediate (16)	-	-	3	-
Citrulline	-	-	-	4
N-acetyl proline	0.6	0.6	0.6	0.6
Perfume	1	1	1	1
Water to	100	100	100	100

Examples 9 to 12

The following formulations represent creams which can be used in the treatment of baldness.

	% w/w			
	9	10	11	12
Cetyl alcohol polyoxyethylene (10)	4	4	4	4
Cetyl alcohol	4	4	4	4
Mineral oil	4	2	-	-
Paraffin wax	-	2	4	-
Intermediate (17)	-	-	-	4
Intermediate (18)	2	-	-	-
Intermediate (19)	-	2	-	-
Intermediate (20)	-	-	2	-
minoxidil	0.5	0.5	0.5	0.5
Triethanolamine	0.75	0.75	0.75	0.75
Butane-1,3-diol	3	3	3	3
Xanthan gum	0.3	0.3	0.3	0.3
Preservative	0.4	0.4	0.4	0.4
Perfume	q.s.	q.s.	q.s.	q.s.
Water to 100		100	100	100

Example 13

This Example illustrates a water-in-oil high internal phase emulsion containing an amine according to the invention.

The emulsion consisted of 10% by volume oily phase and 90% by weight aqueous phase.

The oily phase and the aqueous phase had the following constitution:

	% w/w
Oily phase	
Sorbitan monooleate	20
Quaternium-18 hectorite	5
Liquid paraffin	75
Aqueous phase	
Intermediate (21)	1
Xanthan gum	1
Preservative	0.3
Perfume	q.s.
Sodium chloride (1% w/w solution)	to 100

The emulsion was prepared by taking 10 parts by volume of the oily phase and to it adding slowly with stirring 90 parts by volume of the aqueous phase.

The high internal phase water-in-oil emulsion so formed can be applied topically to the scalp, to improve hair growth and regrowth.

The following two examples 14 and 15 illustrate shampoos for use in washing the hair and scalp, and for promoting hair growth on the scalp.

Example 14

	% w/w
Sodium lauryl ether sulphate (2 EO) [21% AD]	41.4
Lauryl dimethylamino acetic acid betaine: [30% AD]	4
Coconut fatty acid diethanolamine	1.5
Oleyl triethoxy phosphate (BRIPHOS 03D)	1
Polyglycol-polyamine condensation resin (POLYQUART H) [50% active]	1.5
Preservative, colouring matter, salt	0.58

Intermediate (22)	10
Perfume	q.s.
Water	to 100

Example 15

	% w/w
Sodium lauryl ether sulphate (2 EP) [100% AD]	12
POLYMER JR400	2.5
BRIPHOS 03D	2.5
Intermediate (23)	15
Magnesium Sulphate	5
Perfume	q.s.
Water	to 100

Example 16

This Example also illustrates a lotion which is suitable for topical application to the scalp.

The lotion has the following formulation:

	% w/w
Intermediate (24)	5
minoxidil	1
propan-2-ol	10
ethanol	84

Example 17

This example illustrates a powder composition according to the invention which can be applied topically to the scalp.

	% w/w
Chemically modified starch	5
Chemically modified cellulose	-

Boric acid	10
Zinc oxide	5
Intermediate (25)	3
Minoxidil	5
Perfume	q.s.
Chalk	10
Talc	to 100

Example 18

The following example illustrates a lotion according to the invention which can be applied topically to the scalp to prevent hair loss and stimulate hair regrowth.

	% w/w
Intermediate (26)	7
glucaro-1,4-lactone	2
ethanol	16
citric acid	1.05
water	to 100

pH adjusted to 4.2 with sodium hydroxide

Examples 19 and 20

These examples illustrate hair tonics which are suitable for application to the hair and scalp.

The hair tonics had the following formulation:

	% w/w	
	19	20
Intermediate (27)	-	2
Intermediate (28)	2	-
glucaro-1,5-lactam	3	3
ethanol	50	50
water	45	45

Example 21

This example illustrates a shampoo which is suitable for topical application to hair in order to cleanse it, at the same time delivering a metabolic intermediate to the scalp to enhance hair growth or regrowth.

The shampoo had the following formulation:

	% w/w
Triethanolamine lauryl sulphate	16.8
Coconut diethanolamide	3.0
Hydroxypropylmethyl-cellulose (1)	0.25
Corn syrup (80% solids) (2)	20.5
Dimethylpolysiloxane (3)	1.0
Cationic cellulose (4)	0.5
Ethyl alcohol (SDA 40)	9.0
Vinyl carboxy polymer (5)	0.75
Intermediate (29)	8
Perfume, colour, preservative	q.s.
Water	to 100
Acid or base to pH:	6.5

1 - Methocel E4M (Dow Chemical)
2 - 42 Dextrose equivalent (Staley 1300)
3 - 60,000 centistokes (Viscasil, GEC)
4 - Polymer JR 400
5 - Carbopol 941 (BF Goodrich)

Example 22

This example illustrates a shampoo in accordance with the invention.

The shampoo had the following formulation:

	% w/w
Sodium lauryl ether sulphate (3EO)	10
Pearlising agent	4
Betaine	2
Cationic polymer	0.2
Intermediate (30)	6.5
minor ingredients	4
water	to 100

pH value 6 to 7

viscosity: 3500 to 4000 cps (Brookfield Spindle No. 3 at 10 rpm 25°C)

Example 23

This example illustrates a shampoo in accordance with this invention.

The shampoo had the following formulation:

	% w/w
Sodium lauryl ether sulphate [3EO] (70%AD)	20
Pearlising agent	2
Betaine	6
Intermediate (31)	7
glutamine	1
Silicone emulsion	1
Cationic polymer	0.1
D-panthenol	0.4
Carbopol	0.4
Sodium chloride	2.5
Minor ingredients	8.5
Water	to 100

pH value 6.5

viscosity: 5000 cps (Brookfield Spindle No. 3 at 10 rpm, 25°C)

Example 24

This example illustrates a shampoo in accordance with this invention.

The shampoo had the following formulation:

	% w/w
Sodium lauryl ether sulphate [3EO] (70%AD)	20
Pearlising agent	2
Betaine	6
Intermediate (32)	7
glutamine	1
Silicone emulsion	1
Cationic polymer	0.1
D-panthenol	0.4
Carbopol	0.4
Sodium chloride	2.5
Minor ingredients	8.5
Water	to 100

pH value 6.5

viscosity: 5000 cps (Brookfield Spindle No. 3 at 10 rpm, 25°C)

The following examples 25 and 26 illustrate shampoos for use in washing the hair and scalp, and for promoting hair growth on the scalp.

Example 25

	% w/w
Sodium lauryl ether sulphate (2 EO) [21% AD]	41.4
Lauryl dimethylamino acetic acid betaine: [30% AD]	4
Coconut fatty acid diethanolamine	1.5
Oleyl triethoxy phosphate (BRIPHOS 03D)	1
Polyglycol-polyamine condensation resin (POLYQUART H) [50% active]	1.5
Preservative, colouring matter, salt	0.58
Intermediate (33)	10
Perfume	q.s.
Water	to 100

Example 26

	% w/w
Sodium lauryl ether sulphate (2 EP) [100% AD]	12
POLYMER JR400	2.5
BRIPHOS 03D	2.5
Intermediate (34)	15
Magnesium Sulphate	5
Perfume	q.s.
Water	to 100

Example 27

This Example also illustrates a lotion which is suitable for topical application to the scalp.

The lotion has the following formulation:

	% w/w
Intermediate (35)	5
minoxidil	1
propan-2-ol	10
ethanol	84

Example 28

This example illustrates a powder composition according to the invention which can be applied topically to the scalp.

	% w/w
Chemically modified starch	5
Chemically modified cellulose	-
Boric acid	10
Zinc oxide	5
Intermediate (36)	3
Minoxidil	5
Perfume	q.s.
Chalk	10
Talc	to 100

Example 29

The following example illustrates a lotion according to the invention which can be applied topically to the scalp to prevent hair loss and stimulate hair regrowth.

	% w/w
Intermediate (37)	7
glucaro-1,4-lactone	2
ethanol	16
citric acid	1.05
water	to 100

pH adjusted to 4.2 with sodium hydroxide

Example 30

This example illustrates a shampoo which is suitable for topical application to hair in order to cleanse it, at the same time delivering a metabolic intermediate to the scalp to enhance hair growth or regrowth.

The shampoo had the following formulation:

	% w/w
Triethanolamine lauryl sulphate	16.8
Coconut diethanolamide	3.0
Hydroxypropylmethyl-cellulose (1)	0.25
Corn syrup (80% solids) (2)	20.5
Dimethylpolysiloxane (3)	1.0
Cationic cellulose (4)	0.5
Ethyl alcohol (SDA 40)	9.0
Vinyl carboxy polymer (5)	0.75
Intermediate (38)	8
Perfume, colour, preservative	q.s.
Water	to 100
Acid or base to pH:	6.5

1 - Methocel E4M (Dow Chemical)
2 - 42 Dextrose equivalent (Staley 1300)
3 - 60,000 centistokes (Viscasil, GEC)
4 - Polymer JR 400
5 - Carbopol 941 (BF Goodrich)

Example 31

This example illustrates a shampoo in accordance with the invention.

Firstly a pre-emulsion of polyisobutylene was made by mixing at room temperature the following ingredients in a Winkworth twin-Z-blade mixer (model MZ150).

Pre-emulsion ingredient	% w/w
Vistanex[1]	58
Alipal CO-433[2]	1.4
Water	40.6

(1) Polyisobutylene, approx molecular weight 60,000, ex Exxon.
(2) Sodium alkyl phenyl polyethoxylene sulphate.

The pre-emulsion was then mixed, with stirring, with the additional ingredients of the shampoo to give a final composition as follows:

Ingredient	% w/w
Pre-emulsion	1.6
Sodium lauryl ether sulphate 2EO	14
Cocoamidopropylbetaine	2
Euperlan PK900[3]	10
Jaguar C13s[4]	0.1
Citrulline	4
Formalin	0.05
BY 22-026[5]	0.4
NaCl	5
Perfume, colouring	qs
Water	to 100

(3) Mixture of triethylene glycol distearate and SLES 2EO, ex Henkel.

(4) Guar hydroxypropyl trimonium chloride, ex Meyhall.

(5) Polydimethylsiloxane, 50% aqueous emulsion, ex Toray Silicone.

Example 32

	% w/w
SLES 2EO	16
Cocoamidopropyl betaine	2
Silicone emulsion(1)	4
Jaguar C13s	0.1
Ornithine hydrochloride	5.5
Ethylene glycol distearate	5.0
Octopirox(2)	0.5
Preservative, Colour, Perfume	qs
Water	to 100

(1) BY22-026 ex Toray Silicone Co Ltd comprising:

	% w/w
Lauryl alcohol ethoxylate 2EO	2
Lauryl alcohol ethoxylate 21EO	2
Polydimethylsiloxane (60,000 cS)	50
Preservative	qs
Water	to 100

(2) Piroctone olamine ex Hoechst.

The shampoo is prepared using a simple hot process whereby all the ingredients except perfume are mixed at 70°C using a paddle stirrer. The mixture is then cooled slowly, and perfume added below 40°C.

Example 33

	% w/w
Ammonium lauryl sulphate/ Ammonium lauryl ether sulphate	8
Cocodiethanolamide	3
Arginine hydrochloride	5.5
Ionol butyl hydroxytoluene (BHT)	0.05
BRIPHOS 03D(1)	1.05
Hydrolised silk protein	0.1

CARBOPOL 1342[2]	0.4
Polyethoxylated lanolin	0.3
JAGUAR C13s	0.3
TIMIRON MP-1005[3]	0.06
Triethanolamine	0.8
Silicone emulsion (50%)[4]	6
Perfume, Preservative	qs
Water	to 100

(1) A mixture of esters of phosphoric acid and the polyethylene glycol ether of oleyl alcohol.

(2) A copolymer of a carboxylic acid containing monomer and acrylic esters ex Goodrich.

(3) Titanium dioxide coated mica ex Merck.

(4) Silicone emulsion comprises 50% by weight silicone oil (60,000 cs), 4% by weight cetostearyl alcohol and 25% by weight SLES 2EO.

The ammonium lauryl sulphate/ammonium lauryl ether sulphate and the BHT were heated in the main vessel to 75°C to melt the BHT, with constant stirring.

CARBOPOL 1342 was dispersed with stirring in 50% of the water, and the resulting dispersion added to the main vessel.

CLAIMS:

1. Use for increasing, enhancing or maintaining mammalian hair growth, following topical application, of a composition which comprises

(i) an effective amount of a metabolic intermediate of the urea cycle selected from the group consisting of the amino acids arginine, ornithine, citrulline and arginosuccinate, ester, alkyl, acyl, phosphatyl and peptide derivatives of said amino acids and salts and hydrosalts of said aminoacids and derivatives thereof, and

(ii) a cosmetically acceptable vehicle for said metabolic intermediate.

2. Use according to claim 1 wherein the metabolic intermediate is selected from the group consisting of arginine, said derivatives of arginine, and salts and hydrosalts thereof.

3. Use according to claim 2 wherein the metabolic intermediate is selected from L-arginine and L-arginine hydrochloride.

4. Use according to claim 1 wherein the metabolic intermediate is selected from the group consisting of ornithine, said derivatives of ornithine, and salts and hydrosalts thereof.

5. Use according to claim 1 wherein the metabolic intermediate is selected from the group consisting of citrulline, said derivatives of citrulline, and salts and hydrosalts thereof.

6. Use according to claim 1 wherein the metabolic intermediate is selected from the group consisting of arginosuccinate, said derivatives of arginosuccinate, and salts and hydrosalts thereof.

7. Use according to claim 1, wherein the metabolic intermediate is chosen from:

arginine hydrochloride
ornithine hydrochloride
sodium arginate
arginine phosphate, sodium salt

sodium arginosuccinate
potassium arginosuccinate
L-α-alanylarginine hydrochloride
L-cystinylornithine
L-methionylcitrulline
L-arginylarginine
L-ornithylcitrulline
L-glutaminylarginine
L-glutaminylcitrulline
L-tyrosinylarginine
L-arginylmethionine
L-ornithylmethionine
L-citrullylmethionine
L-arginylaspartate
L-arginylglutamate
L-arginylleucine acetate salt
L-arginyllysine acetate salt
L-arginylphenylalanine acetate salt
L-ornithylaspartate
methylarginine dihydrochloride
arginine ethyl ester dihydrochloride
ethylcitrulline
n-propylornithine
n-octylarginine
n-octylcitrulline
n-octylornithine

8. Use according to any one of claims 1 to 7 wherein the composition contains from 0.01 to 30% by weight of said metabolic intermediate.

9. Use according to any one of claims 1 to 8 wherein the composition contains not more than 4% by weight of surfactant.

10. Use according to any one of claims 1 to 8 wherein the composition contains at least 4% by weight of surfactant and more than 5% by weight of said metabolic intermediate or derivative thereof.

11. Use according to any one of claims 1 to 10 by topical application to the bald or balding human scalp.

12. A composition according to claim 11 wherein the metabolic intermediate is selected from the group consisting of arginine, said derivatives of arginine, and salts and hydrosalts thereof.

13. A composition according to claim 12 wherein the metabolic intermediate is selected from L-arginine and L-arginine hydrochloride.

14. A composition according to claim 11 wherein the metabolic intermediate is selected from the group consisting of ornithine, said derivatives of ornithine, and salts and hydrosalts thereof.

15. A composition according to claim 11 wherein the metabolic intermediate is selected from the group consisting of citrulline, said derivatives of citrulline, and salts and hydrosalts thereof.

16. A composition according to claim 11 wherein the metabolic intermediate is selected from the group

consisting of arginosuccinate, said derivatives of arginosuccinate, and salts and hydrosalts thereof.

17. A composition according to claim 11, wherein the metabolic intermediate is chosen from:

arginine hydrochloride
ornithine hydrochloride
sodium arginate
arginine phosphate, sodium salt

sodium arginosuccinate
potassium arginosuccinate
L-α-alanylarginine hydrochloride
L-cystinylornithine
L-methionylcitrulline
L-arginylarginine
L-ornithylcitrulline
L-glutaminylarginine
L-glutaminylcitrulline
L-tyrosinylarginine
L-arginylmethionine
L-ornithylmethionine
L-citrullylmethionine
L-arginylaspartate
L-arginylglutamate
L-arginylleucine acetate salt
L-arginyllysine acetate salt
L-arginylphenylalanine acetate salt
L-ornithylaspartate
methylarginine dihydrochloride
arginine ethyl ester dihydrochloride
ethylcitrulline
n-propylornithine
n-octylarginine
n-octylcitrulline
n-octylornithine

18. A composition according to claim 11 in which the intermediate forms from 0.01 to 30% by weight of the composition.

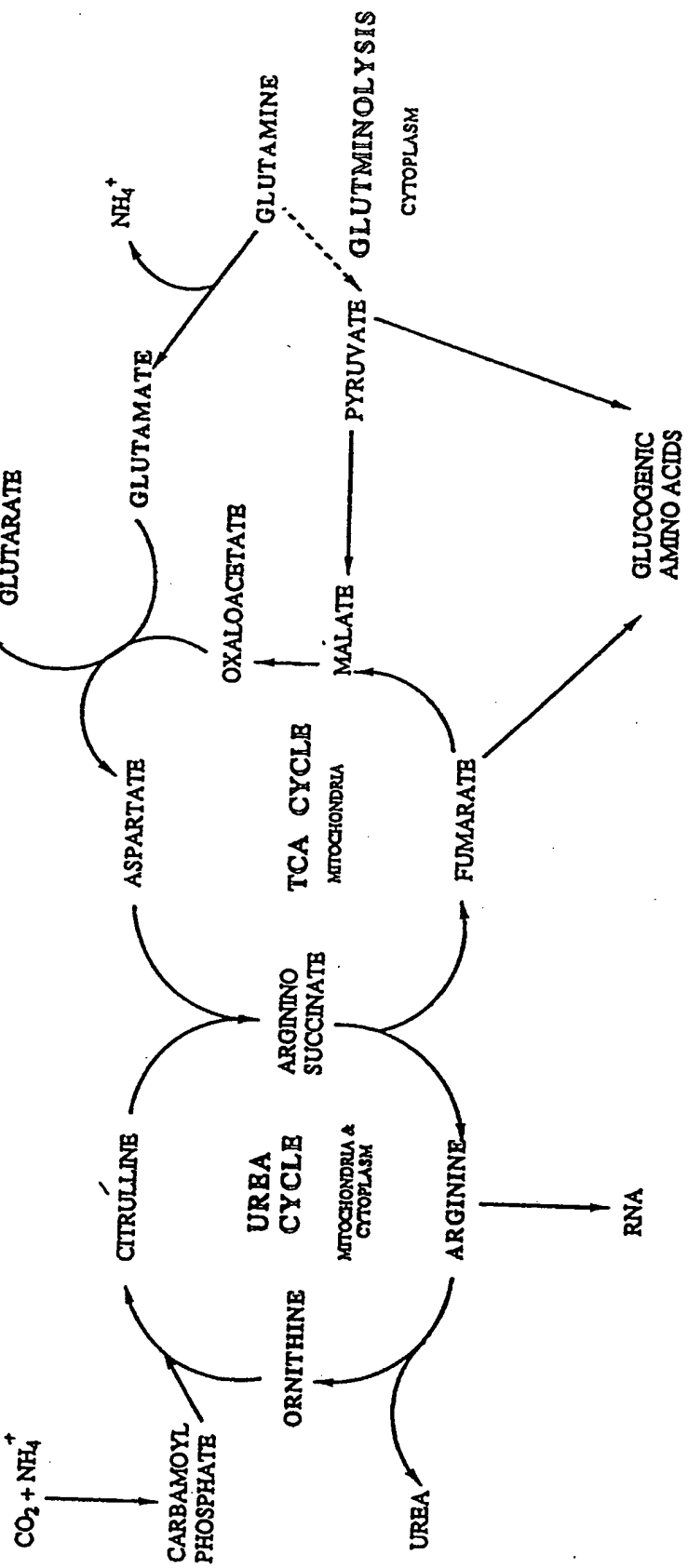

GLUTAMINE
NH_4^+
GLUTMINOLYSIS
CYTOPLASM
GLUTAMATE
PYRUVATE
α KETO GLUTARATE
OXALOACETATE
MALATE
GLUCOGENIC AMINO ACIDS
ASPARTATE
TCA CYCLE
MITOCHONDRIA
FUMARATE
ARGININO SUCCINATE
CITRULLINE
UREA CYCLE
MITOCHONDRIA & CYTOPLASM
ARGININE
RNA
ORNITHINE
$CO_2 + NH_4^+$
CARBAMOYL PHOSPHATE
UREA

FIGURE 1

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/GB 93/02210

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 8634, Derwent Publications Ltd., London, GB; AN 86-221280 & JP,A,61 151 109 (LION CORP) 9 July 1986 see abstract	1,2,4,8, 9,11,12, 14,18
X	GB,A,2 140 297 (LION CORPORATION) 28 November 1984 see the whole document	1,2,4, 9-12,14, 18
X	FR,A,2 669 224 (CONTIER) 22 May 1992 see the whole document	1,2,7-9, 11,12, 17,18

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 February 1994	15.02.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fischer, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE,A,31 18 882 (NORONHA) 5 January 1983 see the whole document	1-3,7-9, 11-13, 17,18
X	GB,A,937 362 (SENDLER-MARBERT) 18 September 1963 see the whole document	1,2,8,9, 11,12,18
E	EP,A,0 571 198 (UNILEVER) see page 12, line 13 - line 55	1,2,4,5, 8,11,12, 14,15,18
X	EP,A,0 336 265 (AJINOMOTO) 11 October 1989 see page 2, line 5 - line 9; claims 1-3; examples 1-7	1,2,4, 8-12,14, 18
P,A	DE,A,42 05 931 (MAINDOK) 26 August 1993 see the whole document	1-18
A	DATABASE WPI Week 8650, Derwent Publications Ltd., London, GB; AN 86-329438 & JP,A,61 246 130 (JAPAN FINE CHEM KK) 1 November 1986 see abstract	1-18
P,A	DATABASE WPI Week 9250, Derwent Publications Ltd., London, GB; AN 92-411554 & JP,A,04 308 523 (KANEBO LTD) 30 October 1992 see abstract	1-18

INTERNATIONAL SEARCH REPORT

...ation on patent family members

Inter...al Application No
PCT/GB 93/02210

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A-2140297	28-11-84	JP-C- 1459157	28-09-88
		JP-A- 59196809	08-11-84
		JP-B- 63005006	01-02-88
FR-A-2669224	22-05-92	NONE	
DE-A-3118882	05-01-83	NONE	
GB-A-937362		NONE	
EP-A-0571198	24-11-93	AU-B- 3870493	25-11-93
EP-A-0336265	11-10-89	JP-A- 1242517	27-09-89
DE-A-4205931	26-08-93	NONE	